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SUMMARY, GENERAL DISCUSSION AND CONCLUSION
SUMMARY

The term ‘chimerism’ originates from Greek mythology and refers to the creature Chimaera (in Greek Χιμαήρα). The front of her body was a lion, the back a serpent, and her midsection a goat. When scientists refer to a chimeric organism, they speak of a single organism composed of at least two genetically different types of tissues. Similarly, in medicine, the term chimerism is used to refer to an individual, organ, or part consisting of tissues of diverse genetic constitution.¹

There are three important sources of chimeric cells; pregnancy, blood transfusion, and transplantation. In Chapter 1 these sources are discussed, and we elaborate on the potential effects of chimeric cells. In this thesis, the role of chimerism was studied in healthy individuals, in solid organ transplant recipients, and in patients who developed the autoimmune disease, Systemic Lupus Erythematosus (SLE).

Part I: Chimerism in healthy individuals

Studies on the prevalence of chimerism in healthy individuals have primarily focused on chimerism during pregnancy. During pregnancy, fetal cells enter the maternal circulation and maternal cells enter the fetal circulation.²⁻⁴ Particularly the former has raised much interest, because fetal DNA can be used for prenatal diagnosti cs.⁵ Furthermore, fetal cells have stem cell-like capacities and can differentiate into several cell types; for example, leucocytes and hepatocytes.⁶⁻⁷ Fetal cells can be detected in the maternal circulation even decades after delivery.⁸⁻⁹ Long-term persistence of fetal chimeric cells in female organs has not been studied extensively, and was the subject of the first study, described in Chapter 2. In situ hybridization (ISH) of the Y chromosome was performed to detect male cells in the organs of 46 women with sons and 29 women without children. Nested PCR of the sex determining region Y (SRY) gene confirmed that the cells identified by in situ hybridization of the Y chromosome were male. Low numbers of male cells were present in 13 of 51 kidneys (25%), 10 of 51 livers (20%), 4 of 69 hearts (6%), and 8 of 44 spleens (18%). Twenty-seven women (36%) had Y chromosome-positive cells present in at least one organ. Six women had male cells in two or three organs. The 27 chimeric women were between 29 and 93 years of age.
No difference was observed in the occurrence of male cells between women with sons and women without children. There was also no correlation between blood transfusion history and the presence of male cells. This study showed that a small number of male cells are present in a significant number of normal female organs. In Chapter 3 we investigate the occurrence of chimeric male cells in normal thyroids, lungs, skin, and lymph nodes of 51 women with sons, using methods similar to those used in Chapter 2. The reason for studying the presence of chimerism in these organs was that these organs were often included in studies investigating the role of chimerism in diseased organs, but normal control organs were scarce. Before conclusions can be drawn regarding the presence and role of chimerism in diseased organs, knowledge of the occurrence of chimerism in normal organs is essential. Male cells were present in 8 of 44 thyroids (18%), 10 of 38 lungs (26%), 3 of 21 skins (14%), and 1 of 7 lymph nodes (14%). Eighteen women (35%) had Y chromosome-positive cells present in at least one organ. Their age ranged from 47 to 81 years. In 4 women, male cells were detected in 2 organs. Also, no relationship between blood transfusion history and the presence of male cells was observed in this study group.

Part II: Chimerism in transplantation

Chimerism in transplantation has been studied extensively, because it may have an effect on transplant outcome. Chimerism after solid organ transplantation can occur as donor-derived cells present in the recipient’s circulation or as recipient-derived cells that replace cells of the allograft. Both situations may improve graft acceptance and tolerance. We reviewed the various methods used to investigate chimerism after transplantation, and the effects of chimerism on outcome. The results are presented in Chapter 4.

When tumors develop in solid organ transplant recipients, the question arises as to whether the tumor cells are of donor or recipient origin. Hematopoietic donor cells are frequently found in the recipient’s circulation and donor-derived cells have been identified in several tissues, raising the possibility of tumors derived from donor origin. In fact, in Kaposi sarcoma, it was demonstrated that tumor cells were indeed of donor origin in a small group of transplanted patients. We investigated whether donor-derived cells are present in skin carcinomas that developed in recipients of a kidney
allograft. As reported in Chapter 5, we did not detect donor-derived cells in basal cell and squamous cell carcinomas of renal allograft recipients, either by in situ hybridization for the Y chromosome or real time quantitative PCR (qPCR) of the Y chromosome-specific gene SRY. The results support that, contrary to endothelial tumors, epithelial cell tumors of the skin in transplant recipients are not donor-derived.

Part III: Chimerism in autoimmune disease

The third subject of this thesis was to study the role of chimeric cells in the pathogenesis of the autoimmune disease, Systemic Lupus Erythematosus (SLE). This chronic, relapsing, inflammatory disorder can affect almost every organ system. Symptoms vary from patient to patient and may come and go resulting in periods of relative quiescence and periods of exacerbations. The hypothesis that chimeric cells may be involved in SLE is based on several observations. Firstly, SLE affects primarily women during the age when they are fertile, and pregnancy is considered the main source of chimerism.\(^{17,18}\) Secondly, fetal cells can survive in the mother’s body for years after pregnancy.\(^8,9\) Thirdly, in a experimental graft-versus-host mouse model, injection of chimeric T cells caused, under certain circumstances, a condition resembling human SLE.\(^9\) We sought to determine the role of chimeric cells in SLE in humans.

The aim of the study described in Chapter 6 was to determine the presence of chimeric male cells in kidneys affected by lupus nephritis. The Y chromosome-positive cells were present in 27 of 49 kidney specimens (55%) which was significantly more frequent than in normal control kidney specimens (25%). The number of male cells was low with a maximum of 5 cells per biopsy slide. Double staining with CD3 and CD34 markers showed that both male T cells (CD3+) and male endothelial/progenitor cells (CD34+) were present. The existence of chimeric T cells in women with SLE is interesting. In the mouse model, the injection of chimeric T cells leads to a graft-versus-host disease that resembles human SLE.\(^9\) However, a graft-versus-host like reaction induced by chimeric T cells is just one hypotheses of the role of chimeric cells in SLE. This hypothesis and two others are discussed in Chapter 7. The first two hypotheses describe the possibility that chimeric cells induce either a graft-versus-host reaction (comparable with reactions seen after bone marrow transplantation) or a host-versus-graft reaction (comparable
with reactions seen after solid organ transplantation). The third hypothesis discusses the possible beneficial role chimeric cells may play in repair mechanisms due to their stem cell-like properties.

To gain more insight into the role of chimeric cells in SLE, we investigated the presence of chimerism in organs either affected or unaffected by SLE. This study is described in Chapter 8. Forty-eight organs from 7 women with SLE were examined for male cells by in situ hybridization of the Y chromosome. Small numbers of male cells were present in all 7 women and in 24 of the 48 organs investigated (50%): 3 of 7 hearts, 4 of 6 lungs, 6 of 7 kidneys, 4 of 7 livers, 2 of 6 spleens, 2 of 6 skins, 2 of 5 thyroids, and 1 of 3 lymph nodes. Chimerism was present more often in organs that had experienced injury, irrespective of whether this injury was SLE- or non-SLE related. Also, no relationship was observed between blood transfusion history and the presence of male cells, or between child-bearing status and the presence of chimerism in this study.

Because it was demonstrated that pregnancy leads to chimerism\textsuperscript{20,21} and since pregnancy is considered the main source of chimerism in women with SLE, we were surprised that we could not detect a relationship between the presence of chimerism and pregnancy in our patients (Chapter 6). A possible explanation for this could be that we did not have complete reproductive data, including miscarriages, of the women included in our study. Because we could use tissue specimens and questionnaires (which included detailed information regarding reproductive data) from women who participated in the First Dutch Lupus Nephritis Study, we were able to investigate this more carefully. In Chapter 9, we detected male cells in 12 of 26 kidney biopsy specimens from women with SLE, of which a complete pregnancy history was known. In this study, there was again no apparent, clear-cut relationship with pregnancy history, although the small number of patients included made small differences difficult to confirm statistically.

Fetal-derived cells enter the maternal circulation during pregnancy via the placenta, and therefore chimerism is expected to be present in uteri, especially shortly after pregnancy. We investigated uteri from women with SLE and healthy controls in Chapter 10. Male cells were identified in the uteri of 7 women with SLE, 11 healthy controls,
and one woman who underwent a hysterectomy directly after the birth of a son. Significantly more chimeric cells were present in the uteri of women with SLE compared with controls. In the uterus removed shortly after delivery due to severe hemorrhage caused by an invasive placenta, even more male cells were present. We hypothesize that abnormal placentation can cause a relatively large influx of chimeric cells. Women with SLE have a significantly increased risk of developing preeclampsia during pregnancy in which several placental abnormalities are present. Reduced invasion of the extravillous trophoblast is most prominent of these abnormalities. Therefore, it may be that the higher occurrence of chimeric cells in women with SLE simply reflects placental pathology.

Finally, in Chapter 11, we evaluate the occurrence of male chimerism in children with lupus nephritis. The occurrence of SLE is higher in adults and affects predominantly women, whereas the incidence between male and female children is almost equal. A possible explanation for this could be that adult women become chimeric more often due to pregnancies. Male cells were present in 12 kidney biopsies from girls with SLE (33%), which was not significantly different from the presence of male cells in the kidney specimens from 11 control girls (36% with male cells). In childhood lupus, Y chromosome–positive cells are probably derived from brothers (transferred from the maternal circulation in utero). Possibly, sibling to sibling chimerism has other immunological consequences besides chimerism from child to mother. The role of maternal chimerism may be more important in children than male chimerism, and should be investigated more extensively before a definitive conclusion on chimerism in juvenile SLE is made.
GENERAL DISCUSSION

Introduction
Chimerism is a frequently occurring phenomenon. Although it was originally believed that the placenta is a natural barrier that prevents an exchange of cells between mother and fetus,\textsuperscript{26} it has now been demonstrated repeatedly that cells from the mother can enter the fetal circulation and cells from the fetus can enter the maternal circulation during pregnancy.\textsuperscript{20,21,27} This indicates that during pregnancy, both mother and fetus become chimeras. Next to pregnancy, blood transfusions and transplantations, either of a solid organ or bone marrow, can also make the recipient chimeric.\textsuperscript{15,28,29} Since the first publication of chimerism,\textsuperscript{30} investigators have been puzzled over the possible roles of chimerism, and whether they are beneficial, harmful, or neutral. In this thesis, we report on the presence and role of chimerism in 3 different groups; namely, healthy individuals, patients who received a solid organ allograft, and patients with the autoimmune disease SLE. The occurrence and role of chimerism differ between these 3 different groups; therefore, they will be discussed separately.

Chimerism in healthy individuals
When discussing the role of chimerism in healthy individuals, pregnant women have an exceptional position. It is known that during pregnancy, both mother and fetus can become chimeras due to the exchange of cells via the placenta.\textsuperscript{20,21,27} This is an interesting finding that raises questions about whether chimerism has a (physiological) role during pregnancy. Several roles of chimerism during pregnancy can be suggested; the presence of fetal cells in the mother’s circulation may be necessary to prevent the mother’s immune system from rejecting the fetus. Another role may be that maternal cells in the fetal circulation accomplish tasks that the fetus cannot perform because of its immaturity, e.g. immune surveillance. In mouse models, fetal cells were shown to be involved in repairing injury induced in the mother during pregnancy.\textsuperscript{31} In humans, these matters still have to be further unraveled. Meanwhile, clinicians have found a practical way to use fetal cells present in the maternal circulation during pregnancy by developing techniques that use fetal DNA for prenatal diagnostic tests.\textsuperscript{5}
After pregnancy, most of the chimeric cells are removed from the circulation of both mother and child. However, in some women, ranging from 13% to 78% in the literature, chimeric cells persist after pregnancy. Apparently, fetal cells may escape elimination by the immune system and survive in the maternal circulation. Whether this is an indication that the host ‘allows’ chimeric cells to be present, or that the chimeric cells are ‘invisible’ to the immune system, is not known. To answer this question, the characteristics of both the chimeric cells and the immune system of the host should be characterized. What is the phenotype of chimeric cells? Can they divide, proliferate, and differentiate like stem cells? Are they immunologically active? Where are chimeric cells located? Are there certain HLA similarities that prevent an immune reaction towards chimeric cells? The answers to all these questions are not yet (completely) known. Investigating these issues is difficult because the number of chimeric cells present is usually low, and sensitive techniques are required to detect and use them for research purposes.

Next to chimerism as the result of pregnancy, chimerism can also be established due to blood transfusion or transplantation. Long-term chimerism caused by blood transfusion seems to occur solely in trauma patients, for which the reason is unknown. Furthermore, in case of multiple donors, most of the persisting chimeric cells originate from a single blood transfusion donor and an in vitro immune response, measured by use of a mixed leucocyte reaction, from the recipient towards the chimeric cells seems very low. This indicates that specific tolerance for these chimeric cells exists. Also, after solid organ transplantation, circulating chimeric cells of donor origin have been demonstrated to persist long-term in the host, and it has been suggested that their presence is necessary for the development of graft tolerance. Chimerism derived from blood transfusions and transplantation is artificially induced, and therefore, a physiological role for this form of chimerism is not expected. However, these cells may interact with cells from the host, thereby influencing the immune system.

To define the role of chimerism, one must first survey in which individuals and in which sites chimerism occurs. Most research on chimeric cells is focused on their presence in blood. In this thesis, we investigate the presence of chimerism in solid tissues, providing
insight into the presence and sites of chimeric cells in both normal and diseased tissues. As described in Chapters 2, 3, and 10, small numbers of chimeric cells are present in heart, lung, kidney, liver, spleen, thyroid, skin, and uteri. Taking the data from these 3 studies together, chimeric cells of male origin were found in at least one organ in 47% of women with sons (25 of the 53 women with sons). The women’s ages ranged from 29 to 93 years. Adding data for women without children, it was concluded that chimerism is present in about 18% of normal organs from women with sons and women without children (59 of 336 organs; Table 1) and in almost half of the women (40 of the 82 women). Interestingly, chimerism was found more frequently in some organs than in others (Figure 1), although the difference was not significant. This finding was also reported in animal studies. The meaning of this result is uncertain, and may be related to several factors including anatomy, cytokine production, cell turnover, injury occurrence, and tissue metabolic rates.

Table 1. Presence of chimerism in normal organs

<table>
<thead>
<tr>
<th>Organ</th>
<th>Women with sons, no./total no. (%)</th>
<th>Women without children, no./total no. (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>6/31 (19)</td>
<td>7/20 (35)</td>
<td>0.32</td>
</tr>
<tr>
<td>Liver</td>
<td>7/34 (21)</td>
<td>3/17 (18)</td>
<td>1.00</td>
</tr>
<tr>
<td>Heart</td>
<td>0/43 (0)</td>
<td>4/26 (25)</td>
<td>0.02</td>
</tr>
<tr>
<td>Spleen</td>
<td>3/30 (10)</td>
<td>5/14 (36)</td>
<td>0.09</td>
</tr>
<tr>
<td>Thyroid</td>
<td>8/44 (18)</td>
<td>n.p.</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>3/21 (14)</td>
<td>n.p.</td>
<td></td>
</tr>
<tr>
<td>Lymph node</td>
<td>1/7 (14)</td>
<td>n.p.</td>
<td></td>
</tr>
<tr>
<td>Uterus</td>
<td>0/5 (0)</td>
<td>2/6 (33)</td>
<td>0.45</td>
</tr>
<tr>
<td>Total</td>
<td>38/253 (15)</td>
<td>21/83 (25)</td>
<td>0.045</td>
</tr>
</tbody>
</table>

Data combined from chapters 2, 3 and 10; n.p., not performed
Categorical variables compared by Fisher’s Exact test

Our data provide a baseline for the occurrence of chimerism in normal organs which can be used for comparison in investigations of diseased or transplanted organs. Furthermore, our data may indicate that in the normal organs investigated, chimeric cells are not harmful. This leaves two other options: chimeric cells could either have a protective role or a bystander role. As an example of a protective role, it has been
suggested that chimeric cells may provide the recipient with a rejuvenating source of cells that can be implemented to replace ‘old’ or damaged cells, thereby maintaining or repairing tissues of the host and reducing tissue destruction.\(^9\) Evidence for a role for chimeric cells in repair has been found in animal studies.\(^{42-45}\) Another protective role may be to modulate the immune system in a positive way through the presence of chimeric cells, thereby preventing certain (immunologically induced) diseases from occurring. Evidence for this hypothesis should come from studies investigating the immunological capacity of chimeric cells. A bystander role is difficult to prove, because its proof relies on the exclusion of all other options.

![Figure 1. Presence of Y chromosome-positive cells (chimerism) in the healthy organs of women with sons. Data from Chapters 2, 3 and 10 combined.](image)

Although the presence of chimeric cells in healthy individuals implies that the chimeric cells have no adverse role in these individuals, it remains interesting why chimeric cells are tolerated by the host. Pregnancy-derived chimeric cells are ‘semi-foreign’ cells, because they have both foreign antigens from the father, and non-foreign antigens from the mother. That chimeric cells can survive in a semi-foreign environment for decades is demonstrated by data from our studies and those of others,\(^{28,46-48}\) and may reflect tolerance of the host towards the chimeric cells. There are various mechanisms by which tolerance can be induced, but a rough distinction can be made between natural tolerance and acquired tolerance. The first is comprised predominantly by the tolerance to self-antigens and is established mainly through the schooling processes
of immature T cells in the thymus. Acquired tolerance can be established by escaping deletion by either activating immune mechanisms that lead to tolerance or by suppressing the immune response that leads to destruction. In a pregnant woman, the woman may develop acquired tolerance toward antigens of the fetus, whereas the fetus develops ‘natural tolerance’ towards its own antigens and probably also towards the mother’s antigens. This may imply that different sources of chimerism have different immunological consequences. Kidney allografts that express HLA-antigens that are similar to the recipient’s mother, but that are not inherited by the recipient (also referred to as non-inherited maternal antigens or NIMAs), are better tolerated than transplants expressing antigens that are similar to the recipient’s father but not inherited by the recipient (referred to as non inherited paternal antigens or NIPAs), leading to better graft survival.49 This may be because the recipient had encountered his or her mother’s antigens before birth due to the materno-fetal circulation during pregnancy and became tolerant of them, but not for those of his or her father.

We surveyed whether there was a relationship between the presence of chimerism and the child’s status and blood transfusion status. Interestingly, we were unable to detect a relationship in any of our studies. Because pregnancy is considered the main source of chimerism, male chimerism was expected to be present more often in women with sons than in women without children. However, sometimes the opposite appeared to be true. Table 1 summarizes the results from all normal women assessed in this thesis. Chimerism was found significantly more often in the organs of women without children than in the organs of women with at least one son. Moreover, we were unable to determine the source of chimerism in some women because they had no living children and had never received a blood transfusion. Chimeric cells in these women may have been the result of unrecognized pregnancies or abortions. This has been attributed to the observation that the termination of pregnancy seems to be the moment of cell exchange.50 An estimated 70% of all pregnancies are lost, of which only 10% are recognized clinically as miscarriages.51 Women with fertility problems who try to become pregnant may experience more pregnancies, although they may be unaware of them, than women with live-birth children.52 These findings suggest that women with fertility problems have a higher chance of becoming chimeric, and may
explain why we did not find a relationship between parental status and the presence of chimerism. Other sources should also be considered, like sibling-to-sibling transfusion in utero from a twin brother or an older brother. The finding that not all women with sons had chimeric male cells in their organs could be due to sampling error or to the phenomenon that only specific chimeric cells can persist long-term in the host, similar to the findings described after blood transfusion.29

In conclusion, in this thesis we demonstrate that chimerism is not restricted to the blood of healthy individuals, but can also occur in healthy hearts, lungs, livers, kidneys, spleens, skins, and thyroids, and that chimeric cells are present in a large proportion of women (in our studies, 50% of all healthy women had male cells in at least one organ).

A direct relationship between parental status and blood transfusion history could not be found, and may be explained by spontaneous abortions or terminated pregnancies (unrecognized pregnancies or elective abortions), the presence of other sources of chimeric cells (e.g. blood transfusion) and the persistence of only certain chimeric cells.

**Chimerism in transplantation**

Chimerism is a frequent subject in transplantation studies, mainly because of the hypothesis put forth by Medawar that when donor cells in an allograft are replaced by cells of the recipient, the graft becomes more ‘self’ to the recipient and therefore rejection is less likely to occur.53 An organ transplant becomes more ‘self’ due to the replacement of damaged or old cells as a result of maintenance or repair after injury. If an allograft’s being more ‘self’ leads to longer survival, the replacement of donor cells by recipient cells as quickly as possible after transplantation would be favorable. One way to establish a quick replacement would be massively damage donor cells in the allograft; for example, as a result of acute rejection. This hypothesis implies that the occurrence of acute rejection leads to more chimerism of the allograft, making the allograft more self-like, thereby preventing the occurrence of chronic rejection which may possibly lead to longer survival of the allograft. One important study by Lagaaij and colleagues54 indicated that the first part of this hypothesis was correct, because they found that the highest percentage of chimerism was in renal allografts in which vascular rejection had occurred.
Studies investigating the relationship between the presence of chimerism in allografts and allograft outcome are scarce, but preliminary results do not show a relationship. Recent studies show that chimeric cells in transplanted organs are not as extensively present as was assumed many years ago when Medawar suggested chimerism might explain graft tolerance. Next to a possible protective role of chimerism in allografts, it has been hypothesized that chimerism may have no influence on allograft survival, but that chimeric cells are just innocent bystanders. Interestingly, studies investigating chimerism in allografts also found that not only was the direct contact area between donor and recipient, the endothelium, able to become chimeric, but chimerism was also found in the parenchymal cells of transplanted organs. In contrast to chimerism in the endothelium, the significance of chimerism in epithelial cells in allografts is more difficult to understand and a good hypothesis has not yet been formulated.

Most studies investigating the presence of chimerism in allografts use detection methods based on sex differences between donor and recipient (i.e. female allografts transplanted into a male recipient). Manual scoring of slides after (fluorescence) in situ hybridization of the Y chromosome is time-consuming, but takes less time than automated scanning, and both techniques have comparable accuracies. The data presented in Chapter 2 show that a small number of male cells are present in a significant number of female organs. Theoretically, these organs could have been used for transplantation. Future studies investigating the role of chimerism after transplantation must take into account that small numbers of chimeric cells may already be present in the organ before transplantation. This indicates that only studies that investigate recipient-derived chimerism in allografts, by using methods that specifically detect cells of the recipient (e.g. with HLA detection techniques), can demonstrate the true origin of donor chimeric cells. However, new techniques should be developed with higher specificity for determining the source of the detected chimeric cells, and have a higher sensitivity to ensure that all chimeric cells present are detected. Only then can we investigate whether chimerism is important for improving graft survival.

Next to the replacement of donor cells in the transplanted organ, chimerism can also be present in the recipient’s body. Donor-derived cells can be detected in the recipient’s
circulation and organs. Persisting donor cell chimerism might be related to donor-specific tolerance or hyporesponsiveness. However, these foreign cells in the recipient may also have a pathogenic role. Previously, in a small group of allograft recipients who developed Kaposi sarcomas, it was determined that the tumor cells were of donor origin. In this thesis, we investigate whether donor-derived cells are present in squamous cell or basal cell carcinomas that develop in the recipient after renal transplantation. We found no evidence of donor-derived cells being involved in the pathogenesis of skin carcinomas after transplantation.

In conclusion, it is important that investigators who wish to investigate the role of chimerism after transplantation consider the possibility that chimeric cells may already be present in the allograft before transplantation. This has implications for the methods used to detect chimeric cells after transplantation. Furthermore, the exact role of chimeric cells with respect to graft survival is unclear, either with respect to recipient cells that replace cells in the allograft, and donor cells that are present in the recipient. However, chimerism is expected to influence tolerance; but the role of chimerism on graft survival may be overruled by other factors involved in graft acceptance.

**Chimerism in autoimmune disease**

In 1996, Lee Nelson suggested that chimerism may play a role in the induction of autoimmune diseases based on the similarities she observed between autoimmunity and graft-versus-host disease after transplantation. Since then, many studies investigating the presence of chimerism in patients with autoimmune diseases have been published. In this thesis, we focus on the autoimmune disease, SLE. In the 1970s, an experimental mouse model was described in which the induction of chimerism in certain mouse strain combinations led to a disease that very much resembled human SLE, with proliferative glomerulonephritis and the presence of anti-dsDNA antibodies.

Before the starting the investigations described in this thesis, there were only a limited number of studies on the presence of chimerism in women with SLE. Abbud-Filho et al. demonstrated that male chimerism was present significantly more often in the blood of women with SLE who had sons (68%) compared with controls who had...
sons (33%). They also observed a tendency toward more chimerism being found with increasing time after giving birth to the youngest son, suggesting proliferation of chimeric cells. Three other studies also investigated the presence of chimerism in the blood of women with SLE with variable results. Mosca et al. did not find a difference between women with SLE and healthy controls. They did observe, interestingly, the highest amount of chimerism in the blood of women with lupus nephritis. In another study, no chimerism at all was found in the blood of 10 women with SLE. Gannagé et al. detected chimerism in 15 women with SLE without sons and 9 women with SLE who had sons. Next to chimerism in the blood of women with SLE, chimerism had also been investigated in the tissues of women with SLE in two studies. Results in tissues also varied. Khosrotehrani et al. investigated 7 skin biopsies from women with SLE, but none were chimeric. They also found no chimerism in skin biopsies of the disease controls. Johnson and colleagues investigated several organs from one woman with SLE who died, and found chimerism in her kidneys, intestines, lungs, heart and skin. No chimerism was found in her thyroid and spleen.

In this thesis, we tried to gather more data on the occurrence of chimerism in the organs of women with SLE and to formulate hypotheses on the possible role of chimerism in SLE. In the studies described in Chapters 6, 8, 9, and 10, we demonstrate that chimerism may be present in every type of organ of women with SLE, that chimerism is present more often in organs of women with SLE than in the organs of healthy control women, and that the occurrence of chimerism is related to tissue injury. These findings raise the question as to whether there is a role for chimerism in SLE, and if so, if this is a beneficial or a harmful role.

In Chapter 7, we postulate three different hypotheses on the possible role of chimerism in SLE. In the first, we postulate that chimerism induces SLE via a graft-versus-host reaction that is similar to the reaction that sometimes occurs after stem cell transplantation. This hypothesis is the basis of the reaction seen in the experimental mouse model, in which an SLE-like disease is induced. To investigate the plausibility of this hypothesis, we investigate whether the chimeric cells present in the kidney biopsies of women with SLE are T cells, and found that, indeed, chimeric T cells were present. From a study by
Scaletti et al.,72 chimeric T cells were found to react against maternal host cells, a finding that favors this hypothesis. In the second hypothesis, chimerism is the target of a host-versus-graft reaction, similar to the reaction that sometimes occurs after solid organ transplantation. Our findings that chimerism is present in so many different organs from women with SLE that are affected by the disease favors this hypothesis. In both of the discussed hypotheses, chimerism is considered to be pathogenic. In the third hypothesis, chimerism is considered to have a beneficial role in SLE; namely, that chimeric cells may replace cells damaged by the autoimmune disease. To investigate this hypothesis, we first determined whether chimeric progenitor cells (CD34+) were present in the kidney biopsies of women with SLE, and secondly, whether there was a relationship with experienced injury. We found chimeric CD34+ cells to be present, indicating that chimeric cells may be stem cells that can differentiate into several different types of cells with different phenotypes. We also observed a relationship between the presence of chimerism and experienced injury. Interestingly, this was irrespective of whether the injury experienced was SLE-related, non-SLE-related, or both.

The chimeric cells that we found in the tissues of women with SLE sometimes seemed to clearly be parenchymal cells (Figure 2; chimeric cardiomyocyte in the heart tissue of a woman with SLE). However, the absence of clusters of chimeric cells can be seen as an argument against massive cell replacement after injury. Another possibility is that chimeric cells are an epiphenomenon in women with SLE. The higher occurrence of chimeric cells in women with SLE compared with control women could be explained by, for example, a reduced immunological capacity of women with SLE to remove the chimeric cells from their bodies, or to a higher overall cell density in the (affected) tissues of women with SLE that lead to higher detection of chimeric cells when a random distribution of chimeric cells throughout the body is assumed.

Although the studies performed in this thesis increase our knowledge about the presence, occurrence, and phenotypes of chimerism in the tissues of women with SLE, we still cannot conclude which hypothesis is more likely to be true. One reason for this is that these three hypotheses are not necessarily mutually exclusive, but could coexist. For example, chimeric cells may be innocent bystanders initially, but may become
pathogenic under certain circumstances. Another reason is that a heterogenic pool of chimeric cells may be present in the host, some of which may be beneficial and some of which may be harmful. For example, chimeric stem cells may play a protective role by helping repair tissues, but chimeric T cells may be pathogenic by inducing a graft-versus-host response. Because SLE is relatively rare, and since there is a relatively high occurrence of chimerism in a wide range of individuals (e.g. in adults of all ages, and in those with and without disease), the presence of chimerism alone is probably not sufficient to induce SLE.

Figure 2. Y chromosome-positive cell in the cardiac tissue of a woman with SLE.

In conclusion, chimerism is present in many women with SLE and in different organ types. It is still unknown whether chimeric cells have a disease-inducing role, a role in repair, a bystander role, or a combination of these. In theory, chimeric cells may not generally be harmful to the recipient, but under certain immunological circumstances, depending on the characteristics of the chimeric cells and of the recipient, chimeric cells become pathogenic. Future investigations should focus on unraveling the characteristics of both chimeric cells and recipients that may lead to autoimmunity.
CONCLUSION

In this thesis, we explored the occurrence of chimerism in the tissues of women, and found chimerism in both healthy and diseased organs and in every organ type investigated. An increased occurrence of chimerism was observed in the organs of adult women with SLE. The long term persistence of chimeric cells that are (semi)allogenic to the host indicates that chimerism may influence tolerance, which could be an interesting study subject for future research in relation to pregnancy, transplantation, and immune-mediated diseases. This topic of research would further unravel the role of chimeric cells. The frequent occurrence of chimeric cells in healthy organs indicates that chimeric cells need not necessarily be harmful. Currently, we believe that under certain circumstances, originally innocuous chimeric cells may become pathogenic and lead to autoimmune disease such as SLE.
REFERENCE LIST


